VIA MEDICA

REVIEW ARTICLE

Alice Indini[1], Fausto Roila[2], Rossana Gueli[1], Francesco Grossi[1, 3], Mario Mandalà[2]

[1]Division of Medical Oncology, Department of Medicine and Surgery, Ospedale di Circolo e Fondazione Macchi, ASST dei Sette Laghi, Varese, Italy
[2]Unit of Medical Oncology, Department of Surgery and Medicine, University of Perugia, Perugia, Italy
[3]Faculty of Medicine and Surgery, University of Insubria, Ospedale di Circolo e Fondazione Macchi, Varese, Italy

Targeted therapy for advanced cutaneous melanoma

Address for correspondence:
Prof. Mario Mandalà, M.D.
Unit of Medical Oncology University of Perugia, Perugia, Italy
e-mail: mario.mandala@unipg.it

Oncology in Clinical Practice
DOI: 10.5603/OCP.2022.0005

ISSN 2450–1654
e-ISSN 2450–6478

ABSTRACT

Drugs targeting the mitogen-activated protein kinase (MAPK) pathway with BRAF and MEK inhibitors have significantly improved survival outcomes of patients with melanoma harboring *BRAF* V600 mutations. To date, three combination targeted therapies have been approved, based on the results of four randomized phase-III trials (COMBI-D, COMBI-V, CoBRIM, and COLUMBUS). In these trials, combined BRAF and MEK inhibitors demonstrated superiority as compared with BRAF inhibitor monotherapy and showed quite homogeneous data in terms of response rate (63%-70%), OS (median > 24 months), and PFS (median values ranging from 11 to 14 months). Consequently, different toxicity profiles of each combination therapy presently help with the decision-making process. Despite these successful results, treatment resistance represents an issue during both immunotherapy and targeted therapy, and there is presently no consensus on the therapeutic journey of patients with *BRAF* mutant melanoma to optimize their survival results. Several strategies to further increase therapeutic results of targeted therapy have been investigated, by combining and/or sequencing different treatment approaches. In this review, we will present the molecular features of cutaneous melanoma, focusing on *BRAF* mutation, the therapeutic rationale of targeted therapies, their efficacy, and toxicity, and give an overview of future perspectives in the treatment of this disease.

Key words: BRAF, MAPK, melanoma, metastatic disease, targeted therapies

Oncol Clin Pract 2022; 18, 3: 197–207

Introduction

Before the advances in the treatment of advanced/metastatic melanoma [i.e., unresectable stage III/stage IV disease according to the American Joint Committee on Cancer (AJCC) staging system, 8th edition], disease outcomes with chemotherapy were very poor [1]. Historically, patients with advanced disease had median overall survival (OS) of approximately 7.5 months and a 5-year survival rate of ~6% [1]. Over the last decades, two therapeutic strategies have significantly improved survival outcomes of patients with metastatic melanoma. The first one involves modulating the immune system with monoclonal antibodies acting as immune-checkpoint inhibitors (ICIs), targeting the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), or the programmed cell-death 1 (PD-1) [2–4]. The second class of drugs targets the mitogen-activated protein kinase (MAPK) pathway, which is constitutively active in melanomas harboring *BRAF* V600 mutations [5]. To date, targeted therapy with BRAF and MEK inhibitors represents the first choice of treatment for most patients with *BRAF* mutant melanoma due to the impressive survival results obtained in certain settings (e.g., patients with a low tumor burden). Several strategies to further increase therapeutic results of targeted therapy have been investigated by combining and/or sequencing different treatment approaches. Still, treatment resistance

Received: 26.01.2022 Accepted: 26.01.2022 Early publication date: 22.02.2022

represents an issue during both immunotherapy and targeted therapy, and there is presently no consensus on the therapeutic journey of patients with *BRAF* mutant melanoma to optimize survival results.

In this review, we will present the molecular features of cutaneous melanoma, focusing on *BRAF* mutation, the therapeutic rationale of targeted therapies, their efficacy, and toxicity, and give an overview of future perspectives in the treatment of this disease.

Molecular features of cutaneous melanoma

Based on the pattern of the most prevalent significant mutated genes in cutaneous melanoma, the Cancer Genome Atlas Network (TCGA) performed a multi-platform characterization of 333 cutaneous melanomas at the DNA, RNA, and protein levels, creating a framework for genomic classification with four subtypes: mutant *BRAF* (with an incidence of 52%), mutant *RAS* (28%), mutant *NF1* (14%), and Triple-wild type [6]. The most common *BRAF* mutation is the V600E, accounting for nearly 90% of mutations, while others are far less common (e.g., V600K, V600D) [7]. Other common genetic alterations found in cutaneous melanoma are *NF1* mutations (15%) and activating mutations of neuroblastoma *RAS* (*NRAS*) (15–30%) [6]. The gain-of-function *BRAF* and *NRAS* and the loss-of-function *NF1* mutations all lead to the constitutive activation of downstream RAS/RAF/MEK/ERK proteins (i.e. the MAPK pathway), which proteins sustain tumor cell proliferation and survival and is a key driver in the pathogenesis of melanoma [8]. However, despite several efforts, no *RAS* inhibitors have yet demonstrated their efficacy in clinical trials [9]. Combinations of BRAF and MEK inhibitors that target the MAPK pathway have been developed. Therefore, only mutations in the $BRAF^{V600}$ gene are therapeutically relevant, while no other valid druggable targets have been identified so far.

Current evidence and future challenges of BRAF and MEK inhibitors

Vemurafenib (PLX4032; trade name: Zelboraf®) was the first molecule to establish the clinical activity of BRAF inhibitors (BRAFi) in *BRAF* mutant melanoma [10]. The BRIM-3 trial was a randomized phase-III clinical trial comparing vemurafenib with dacarbazine in 675 patients with previously untreated $BRAF^{V600E/K}$ mutant metastatic melanoma [11]. The overall response rate (ORR) was 48% and 5%, with vemurafenib and dacarbazine, respectively [11]. Clinical benefit was seen in all enrolled patients, including those with M1c stage and/or the elevated baseline lactate dehydrogenase (LDH) level. Based on the results of this clinical trial, in 2011, vemurafenib was approved by the United States (US) Food and Drug Administration (FDA) for the treatment of $BRAF^{V600}$ mutant advanced melanoma patients. A recently updated analysis of the BRIM-3 trial results showed that Kaplan-Meier estimates of OS rates for vemurafenib *vs.* dacarbazine were 56% *vs.* 46%, 30% *vs.* 24%, 21% *vs.* 19%, and 17% *vs.* 16% at 1, 2, 3 and 4 years, respectively [12].

Dabrafenib (GSK2118436; trade name: Tafinlar®) was the second BRAFi to demonstrate a significantly improved progression-free survival (PFS) in comparison with conventional cytotoxic chemotherapy among patients with $BRAF^{V600E}$ mutant melanoma (5.1 *vs.* 2.7 months for dabrafenib and dacarbazine, respectively) [13]. ORR was 50% and 6% in patients who received dabrafenib and dacarbazine, respectively. Dabrafenib received US FDA approval for the treatment of patients with $BRAF^{V600E}$ mutated melanoma in 2013.

Despite the clinical benefit seen in nearly all patients with *BRAF* mutant melanoma receiving vemurafenib or dabrafenib monotherapy, median PFS lasts only six months and 90% of patients develop resistance within one year from starting treatment [14]. Several acquired molecular mechanisms account for this resistance; however, the most important is the reactivation of the MAPK pathway through alternative activation of downstream MEK [15, 16]. Dual MAPK pathway inhibition with MEK inhibitor (MEKi) plus a BRAFi [17] led to improved efficacy and tolerability of treatment, as reported in the results of Phase-III prospective randomized studies [18–20]. The therapeutic efficacy of the vemurafenib and cobimetinib (GDC-0973; trade name: Cotellic®) combination was first demonstrated in the BRIM-7, open-label, phase-Ib, dose-escalation study [21]. This trial enrolled patients with advanced $BRAF^{V600}$ mutant melanoma who had progressed, or not, on vemurafenib. Treatment consisted of vemurafenib 720 or 960 mg twice a day continuously, and cobimetinib 60, 80, or 100 mg once daily with different schedules of administration (14-days on/14-days off, 21-days on/7-days off, or continuous). The ORR was 87% *vs.* 15%, and PFS was 13.7 and 2.8 months, in vemurafenib naive and pre-treated patients, respectively. Median OS in BRAFi naive population was 31.2 months, and OS at 1, 2, 3, and 4 years was 82.5%, 63.9%, 39.2, and 35.9%, respectively. This study found the safest schedule to be continuous vemurafenib 960 mg twice daily, plus cobimetinib 60 mg daily 21-days on/7-days off, which then became the approved regimen for clinical use.

The subsequent CoBRIM study was the clinical trial that led to the FDA approval of vemurafenib in combination with cobimetinib [18]. In this Phase-III

multicenter trial, patients with previously untreated, locally advanced stage IIIC or IV *BRAF*^{V600} mutant melanoma were randomly assigned to receive vemurafenib plus cobimetinib (n = 247) or vemurafenib plus placebo (n = 248). The ORR was significantly improved with the combination therapy compared to BRAFi alone (70% *vs.* 50%, p < 0.0001). Updated results with an extended follow-up showed that at a median follow-up of 14.2 months, the median PFS was 12.3 for the combination group and 7.2 months for the control group (HR for death or disease progression 0.58, 95% CI 0.46–0.72, p < 0.0001) [22]. Median OS for the combination therapy group was 22.3 months (95% CI, 20.3–not reached) *vs.* 17.4 months (95% CI, 15–19.8) for the monotherapy group (HR 0.70, 95% CI 0.55–0.9, p = 0.005). Combined BRAFi + MEKi confirmed their superiority regardless of baseline prognostic factors, such as the tumor burden or presence of visceral metastases: OS at 1-, 2- and 3-year was 74.5%, 48.3%, and 37.4%, respectively, in the vemurafenib plus cobimetinib group, and 63.8%, 38.0%, and 31.1%, in the control group. Survival results were even better in the subgroup of patients with normal *vs.* elevated LDH levels. A longer PFS was observed with the combination even in patients with *BRAF* ^{V600K} mutant melanoma, which is a rare mutation known to confer less sensibility to BRAFi (HR 0.27) [22].

The pharmacokinetic activity and safety of combined dabrafenib and trametinib (GSK1120212; trade name: Mekinist®) was investigated in an open-label study in 85 *BRAF*^{V600} mutated metastatic melanoma. The same study subsequently randomized 162 patients with *BRAF*^{V600} mutated metastatic melanoma to receive combination therapy with dabrafenib plus trametinib or dabrafenib monotherapy [23]. The median PFS was 9.4 months with the combination *vs.* 5.8 months with monotherapy (HR for progression or death 0.39, 95% CI 0.25–0.62, p < 0.001). The phase-III trial COMBI-d evaluated 423 patients with advanced/metastatic *BRAF*^{V600} mutant melanoma, who were randomly assigned to receive first-line treatment with dabrafenib and trametinib or dabrafenib plus placebo [24]. The ORR was higher with the combination therapy (67 *vs.* 51%, p = 0.002). In the updated analysis, the median duration of progression-free survival was 11.1 months. The PFS rates were 21% at 4, and 19% at 5 years. Patients with a normal baseline lactate dehydrogenase level (at or below the upper limit of the normal range) had a 5-year progression-free survival rate of 25% as compared with 8% in patients with an elevated lactate dehydrogenase level at baseline. In the subgroup of 216 patients with normal LDH levels and fewer than three disease sites at baseline, the 5-year progression-free survival rate was 31% [25]. The median OS duration was 25.9 months, with OS rates of 37% at 4 years, and 34% at 5 years. Similarly, the 5-year OS rate was higher among the patients who had a normal LDH level at baseline than among those with an elevated level (43% *vs.* 16%). The estimated 5-year OS rate was 55% among patients with a normal LDH level and fewer than three organ sites with metastasis at baseline [25]. Importantly, the combination of dabrafenib and trametinib seemed to improve health-related quality of life compared to dabrafenib alone [26].

The efficacy of dabrafenib and trametinib *vs.* vemurafenib alone was evaluated in the phase-III COMBI-v trial [27]. ORR was higher in the dabrafenib plus trametinib arm compared to vemurafenib alone (67 *vs.* 53%, p < 0.001). Median PFS was significantly longer among patients treated with the combination therapy (12.1 *vs.* 7.3 months; HR 0.61, 95% CI 0.51–0.73, p < 0.001); median OS was also improved: 26.1 months and 17.8 months in the combination and monotherapy group, respectively. Consistently, the subgroup of patients with normal baseline LDH levels demonstrated to gain even more benefit from the combination therapy, with a median PFS? of 17.5 months among patients treated with the combination therapy (*vs.* 9.2 months with monotherapy, HR 0.55) while, in the subgroup of patients with elevated LDH levels, median PFS in the combination therapy arm was 5.5 months (*vs.* 4.0 months with monotherapy, HR 0.70). In the subgroup of patients with normal LDH levels, median OS was 21.5 months with vemurafenib alone and median OS was not reached with the combination (HR 0.56) [27]. According to the latest update, the survival benefit was maintained over time: the 2- and 3-year analysis showed that 53 and 45% of patients, respectively, were still alive in the combination therapy group *vs.* 39 and 31% of patients receiving vemurafenib alone [28].

Notably, trametinib was the only MEKi that showed clinical activity as monotherapy in BRAF-mutant melanoma. Based on the results of a phase-II study on BRAFi-naive patients, in which trametinib showed significant clinical activity in patients with *BRAF*-mutant melanoma [29], the phase-III METRIC trial compared first-line treatment with trametinib *vs.* conventional chemotherapy (dacarbazine or paclitaxel) [30]. Patients receiving trametinib demonstrated a higher ORR (22 *vs.* 8%), a longer median PFS (4.8 *vs.* 1.5 months, p < 0.001), and increased 6-month OS (81 *vs.* 67%, HR 0.54, p = 0.01). Based on these results, in 2013, trametinib was approved by US FDA for the treatment of *BRAF* $^{V600E/K}$ mutant melanoma patients not previously exposed to BRAFi.

Recently, a third combination of BRAFi and MEKi has been developed and approved. Combined treatment with encorafenib (LGX818; trade name: Braftovi®) and binimetinib (ARRY-162; trade name: Mektovi®)

extended PFS and reduced the risk of death compared to vemurafenib monotherapy, based on the results of the pivotal two-part, Phase-III randomized COLUMBUS trial [20]. In Part 1, *BRAF*$^{V600E/K}$ mutant metastatic melanoma patients (n = 577) were randomly assigned (1:1:1) to receive encorafenib 450 mg once daily plus binimetinib 45 mg twice daily, or monotherapy with standard-dose vemurafenib, or encorafenib 300 mg once daily. The primary endpoint was the median PFS of the combination *versus* vemurafenib. At the primary analysis (median follow-up: 16.6 months), median PFS was 14.9 months in the combination group, and 7.3 months in the vemurafenib group (HR 0.54, p < 0.0001). ORR was 63% in the combination therapy group, and 40% in the vemurafenib group. At a pre-planned OS analysis, the median OS with encorafenib plus binimetinib was 33.6 months, compared with 16.9 months for vemurafenib alone (HR 0.61, p < 0.0001) [31]. Part 2 of the COLUMBUS trial was conducted upon request of the US FDA, to better understand the contribution of binimetinib in the combination therapy, through comparison of encorafenib 300 mg once daily plus binimetinib 45 mg twice daily *vs.* encorafenib 300 mg daily monotherapy. The second part randomized 344 patients in a 3:1 ratio and is currently ongoing. Preliminary results from a primary analysis of Part 2 showed a longer PFS with combination therapy (n = 258 patients) compared with the encorafenib monotherapy group (i.e. n = 280 patients treated with encorafenib 300 mg in COLUMBUS Parts 1 and 2 combined) [32]. Median PFS was 12.9 *vs.* 9.2 months for the combination and the monotherapy groups, respectively (HR 0.77, p = 0.029) [32]. A five-year update from the Part 1 of the COLUMBUS trial was recently presented, confirming a median OS of 33.6 months and a 5-year OS rate of 34.7% with combination therapy (median follow-up: 70.4 months) [33]. The 5-year OS rate among patients who had normal LDH at baseline and received combination therapy was 45.1%. The 5-year PFS rate for combination therapy, encorafenib monotherapy, and vemurafenib monotherapy was 22.9%, 19.3%, and 10.2%, respectively; ORR was 64.1%, 51.5%, and 40.8%; and the median duration of response (DOR) was 18.6, 15.5, and 12.3 mo, respectively [33].

The four randomized phase-III trials comparing the therapeutic efficacy of the combination of BRAFi and MEKi *vs.* BRAFi alone (COMBI-D, COMBI-V, CoBRIM, and COLUMBUS) showed quite homogeneous data in terms of the response rate (63–70%), OS (median > 24 months), and PFS (median values ranging from 11 to 14 months). The latter reflects the development of resistance mechanisms in the majority of patients. From a molecular point of view, the acquired resistance is related to a re-activation of the MAPK pathway [15–17]. From a clinical point of view, a regression tree analysis identified three independent favorable prognostic factors during treatment with BRAFi plus MEKi: pre-treatment LDH levels, presence of < 3 metastatic sites, and the sum of lesion diameters < 66 mm. In the most favorable prognostic group, 3-year PFS was 42%, suggesting that a low disease burden at baseline can be prognostic for a long-term benefit with targeted therapies [34, 35].

All studies with BRAFi + MEKi continued treatment until disease progression or the onset of unacceptable treatment-related toxicities, which is the standard of care in clinical practice. Experience deriving from small case series in the literature suggests that treatment discontinuation, even after the complete response has been reached, leads to disease recurrence in 50% to 100% of patients [36, 37] and is, therefore, not recommended.

On the contrary, in presence of oligoprogression, targeted therapy can be continued to obtain the best therapeutic results. In retrospective series, it has been reported that the so-called "treatment beyond progression" can increase disease control by adding a loco-regional approach and maintaining the targeted therapy. In a retrospective analysis of 114 patients enrolled in clinical trials, 31% of them progressed in isolated sites [38]. Even after adjusting for potential prognostic factors at progression, continued BRAFi was associated with prolonged OS compared with cessation. In a long-term follow-up analysis of patients treated in the phase-I vemurafenib trial, the median survival was 26.0 months (range, 7.7–56.1) among 20 patients who continued vemurafenib after local therapy [39]. Nevertheless, these retrospective analyses cannot exclude selection biases and different paths of melanoma growth in patients who received (or not) treatment beyond progression.

Table 1 summarizes the outcome and landmark analyses of the available doublet combinations. Currently, the long-term activity and the efficacy of different combo-targeted therapies so far reported seem to be quite similar. Consequently, different toxicity profiles of each combination therapy should drive clinicians in routine activity.

Targeted therapy for the treatment of brain metastases

The activity of dabrafenib as monotherapy and dabrafenib plus trametinib was investigated in melanoma patients with brain metastases. Results from the phase 2 BREAK-MB trial provided evidence that dabrafenib monotherapy exhibits clinical activity and a manageable safety profile in patients with *BRAF*$^{V600E/K}$ mutant melanoma brain metastases, regardless of previous local treatment [40]. The subsequent phase-II COMBI-MB trial investigated the combination of dabrafenib and

Table 1. Overview of the most common adverse events associated with BRAF and MEK inhibitors as monotherapy or in combination in major clinical trials

	Monotherapy				Combination therapy				
Study	**BREAK–3**	**BRIM–3**	**METRIC**	**NEMO**	**BRF112320 (part c)**	**COMBI–d**	**COMBI–v**	**coBRIM**	**COLUMBUS**
Agent(s)	**Dabrafenib**	**Vemurafenib**	**Trametinib**	**Binimetinib**	**Dabrafenib + Trametinib**	**Dabrafenib + Trametinib**	**Dabrafenib + Trametinib**	**Vemurafenib + Cobimetinib**	**Encorafenib + Binimetinib**
Patients (n)	187	336	211	269	55[a]	209	350	247	192
Any AE (%)	–	99	–	–	100	87	98	99	98
Grade 3–4 AEs	–	71	–	–	58	32	48	75	58
Most common AEs (≥ 20% incidence), any grade/grade 3–4(%)									
Pyrexia	33/4	21/< 1	–	10/0	71/5	52/7	53/4	29/1	16/4
Chills	12/0	7/0	–	–	58/2	28/0	31/1	–	–
Fatigue	26/2	46/3	26/4	20/2	53/4	27/2	29/1	37/4	27/2
Nausea	29/< 1	38/2	18/1	28/1	44/2	20/0	35/< 1	42/1	42/2
Vomiting	22/2	21/2	13/1	19/2	40/2	14/< 1	29/1	25/2	30/2
Diarrhea	17/1	36/1	43/0	39/1	36/2	18/< 1	32/1	61/6	35/2
Arthralgia	39/2	56/6	–	–	27/0	16/< 1	24/1	38/2	27/1
Headache	36/0	33/1	–	–	29/0	19/0	29/< 1	–	23/2
Rash	19/0	41/9	57/8	32/4	27/0	24/0	22/1	72/17[b]	27/2[b]
Cough	18/0	13/0	–	–	29/0	–	20/0	–	12/1
Peripheral edema	–	20/< 1	26/1	36/< 1	29/0	11/1	12/< 1	–	10/2
Decreased appetite	13/0	22/< 1	–	11/1	22/0	–	12/< 1	–	9/0
Pruritus	–	25/1	10/2	11/1	–	7/0	9/0	–	12/1
Acneiform dermatitis	–	5/0	19/< 1	33/3	16/0	8/0	6/0	–	–
Alopecia	29/< 1	48/0	17/< 1	–	5/0	5/0	6/0	17/< 1	14/0
Constipation	14/2	14/< 1	14/0	13/1	22/0	–	13/0	–	24/0
Asthenia	20/< 1	14/< 1	–	15/3	–	–	16/1	–	19/2

→

Table 1 cd. Overview of the most common adverse events associated with BRAF and MEK inhibitors as monotherapy or in combination in major clinical trials

	Monotherapy				Combination therapy				
Study	**BREAK–3**	**BRIM–3**	**METRIC**	**NEMO**	**BRF112320 (part c)**	**COMBI–d**	**COMBI–v**	**coBRIM**	**COLUMBUS**
Myalgia	17/0	15/1	–	–	22/2	–	–	–	16/0
Photosensitivity reaction	3/0	41/4	–	–	–	–	4/0	48/4	3/1
cuSCC/KA	12/7	30/29	0	–	7/5	3/3	1/1	6/5	3/1
Dry skin	13/0	23/0	11/0	–	–	9/0	8/0	–	16/0
Hyperkeratosis	41/2[c]	29/1	–	–	9/0	6/0	4/0	10/< 1	15/1
Hand foot syndrome/PPE	20/2	9/< 1	–	–	–	6/< 1	4/0	–	16/0
Skin papilloma	26/0	28/< 1	–	–	4/0	1/0	2/0	–	8/0
Hypertension	–	3/1	15/12	6/7	9/2	–	26/14	–	8/6
Increased ALT	–	8/2	–	6/3	–	10/2	14/3	26/11	6/5
Increased AST	–	7/< 1	–	11/2	11/3	11/1	11/1	24/9	7/2
Increased creatine kinase	–	7/< 1	–	23/19	–	–	–	35/12	18/8
Increased γ-GT	–	–	–	2/1	–	–	–	22/15	6/9
Serous retinopathy[d]	–	–	–	–	2/2	< 1/0	1/0	27/3	–

[a]Data for dosage arm of dabrafenib 150 mg twice daily plus trametinib 2 mg once daily; [b]Combined terms include the preferred terms rash, rash maculopapular, erythema, dermatitis acneiform, folliculitis, rash macular, rash papular, rash erythematous, acne, dermatitis, rash pruritic, furuncle, rash generalized, dermatitis allergic, rash follicular, rash pustular, dermatitis exfoliative, generalized erythema, rash morbilliform, and drug eruption; [c]Hyperkeratosis included acanthoma, acrochordon, actinic keratosis, keratosis pilaris, lichenoid keratosis, papilloma, seborrheic keratosis, and skin papilloma; [d]Combined terms include the preferred terms chorioretinopathy, retinal detachment, detachment of retinal pigment epithelium, macular oedema, macular fibrosis, retinal disorder, retinopathy, subretinal fluid, and detachment of macular retinal pigment epithelium; AE — adverse event; ALT — alanine aminotransferase; AST — aspartate aminotransferase; cuSCC — cutaneous squamous cell carcinoma; γ-GT — gamma-glutamiltransferase; KA — keratoacanthoma; PPE — palmar-plantar erythrodysesthesia

trametinib in four melanoma patient cohorts: (A) *BRAF*^{V600E}, asymptomatic, no prior local brain therapy; (B) *BRAF*^{V600E}, asymptomatic, prior local brain therapy; (C) *BRAF*$^{V600D/K/R}$, asymptomatic, with or without prior local brain therapy; and (D) *BRAF*$^{V600D/E/K/R}$, symptomatic, with or without prior local brain therapy [41]. The primary endpoint was intracranial response rate (IRR), and it was met only in cohort A (IRR 58%). Intracranial responses were observed also in cohorts B, C, and D (IRR 56, 44, and 59%, respectively), but due to the small sample sizes of these cohorts, these findings should be considered exploratory. The median duration of response was relatively short, between 4.2 and 7.2 months [41].

Data from the phase-II trial GEM1802/EBRAIN-MEL, evaluating the combination of encorafenib and binimetinib among two different cohorts of patients with brain metastases (i.e., patients with symptoms and those asymptomatic) showed that this combination provided intracranial response rate of 64.3% and 63.6% in the two patients cohorts, thus supporting clinical activity of targeted therapy regardless of the presence of symptoms [42].

Safety profile and toxicity of BRAFis and MEKis

BRAFis and MEKis display peculiar adverse events (AEs), which are similar in the two classes of drugs while some are specific to a particular drug. Both on-target and off-target AEs have been reported, with on-target AEs being related to the paradoxically hyper-activation of the MAPK pathway. Most AEs are milder with the combination of the two agents, while others exacerbate. Since targeted therapy is taken chronically until disease progression or unacceptable toxicity, prompt identification and treatment of AEs and preservation of quality of life (QoL) are important goals in patients' management [43].

The safety profile of BRAFi and MEKi drugs has been well characterized both in clinical trials and routine clinical practice. The highest rates of AEs seem to occur early in treatment and their incidence decreases over time [43]. Most AEs are mild [i.e. grade 1–2 according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.03], transient, and easily manageable with treatment withdrawal, without requiring dose adjustments. In studies with combination treatment, the incidence of dose reduction or interruption due to AEs range between 11–58% and 46–67%, respectively, while the percentage of patients permanently discontinuing treatment due to AEs was 11–14%. Importantly, BRAFi- and MEKi-related AEs usually resolve with therapy withdrawal and late toxicities are uncommon after drug discontinuation [43].

Each combination displays a peculiar profile of AEs, though most of them are similar and their prevalence varies according to the specific combination. Table 2 summarizes the incidence of AEs reported in the major clinical trials of mono- and combo-targeted therapy. The most common AEs during treatment with vemurafenib and cobimetinib were gastrointestinal (GI) events (i.e. diarrhea, nausea, and vomiting), cutaneous rash, fatigue, pyrexia, arthralgia, photosensitivity reactions, increased creatinine kinase (CK) levels, and altered liver function tests (LFT). Some of those AEs had an increased incidence in the case of the combination compared with BRAFi monotherapy (e.g. GI events, photosensitivity reactions, and altered LFTs). Similarly, combination therapy was characterized by a higher incidence of MEKi-related AEs, such as elevated CK levels and ocular events. Ocular toxicity depends on the inflammatory response and breakdown of the blood-retinal barrier brought driven by MAPK pathway inhibition. AEs can range from mild visual impairment and decreased visual function to more serious uveitis, retinal epithelial detachment, and retinal vein occlusion. The latter effect generally implies the permanent discontinuation of

Table 2. Overview and comparison of the major characteristics of clinical trials of combination targeted therapy for melanoma

Clinical study (reference)	ORR	Median PFS	Median OS	OS (%)			≥ 3 met. sites	LDH > ULN	I-O post	Discontinuation
				1 yr	2 yrs	3 yrs				
COBRIM	70%	12.3	22.3	75%	48%	–	–	46%	18%	16.6%
COMBI-d	68%	11.0	25.1	74%	52%	44%	48%	36%	20%	14%
COMBI-v	67%	12.1	26.1	72%	53%	45%	50%	34%	9%	16%
COLUMBUS	76% 64% BIRC	14.9	33.6	75.5%	57.6%		45%	29%	20%	15% 6% drug related

BIRC — Blinded Independent Review Committee; LDH — lactate dehydrogenase; ORR — objective response rate; OS — overall survival; PFS — progression-free survival; ULN — upper limit of normal

treatment. However, most ocular events are transient and self-limiting and either resolve with dose reduction or temporary drug interruption [44]. Combination therapy had a lower incidence of hyperproliferative cutaneous lesions, which were commonly observed with vemurafenib monotherapy [18, 21–22]. This type of skin toxicity, affecting virtually all patients receiving BRAFi monotherapy, results from the paradoxical activation of the MAPK pathway leading to subsequent keratinocyte hyperproliferation and development of cutaneous squamous cell carcinoma (SCC), verrucal keratosis, and plantar hyperkeratosis [45]. Data from a specific analysis of the characteristics and patterns of AEs in the coBRIM trial indicate that most treatment-related AEs generally occur early in the treatment course, are mild to moderate, and are manageable by patient monitoring, dose modification, and supportive care [43].

The safety profile of dabrafenib and trametinib was evaluated in three clinical trials [19, 24, 27]. The most common AEs were pyrexia, chills, fatigue, headache, GI events (nausea, diarrhea), arthralgia, cutaneous rash, and hypertension. Pyrexia, in particular, was one of the leading reasons for dose modification, treatment interruption, and permanent withdrawal [24]. Also, for dabrafenib and trametinib, MEKi related AEs (i.e. peripheral edema, decreased left ventricular ejection fraction [LVEF], and acneiform dermatitis) were most common with the combination therapy, while hyperproliferative skin lesions were less commonly observed [24].

Data regarding the safety of encorafenib and binimetinib suggest that it might overcome other combination therapies for its tolerability. The most-reported AEs in part I of the COLUMBUS trial were GI events, fatigue, increased CK, and headache [31]. The incidence and severity of pyrexia were much lower than with dabrafenib and trametinib. In the COLUMBUS trial, pyrexia with encorafenib and binimetinib was low in frequency (18%) with few grade 3 events (4%) and resulted in few dose modifications or discontinuations. The majority of the higher grade of adverse events were associated with concurrent infection or progression of the disease. Furthermore, photosensitivity was rarely observed.

Data from phase-III trials suggest that most AEs are manageable with temporary drug interruption, while only intolerable AEs require dose modification and/or discontinuation. Usually, the drug that is most likely associated with an AE should be interrupted and/or reduced. To optimize clinical response while preserving QoL, early detection and management of treatment-related AEs are of paramount importance. Reports from case series of patients interrupting treatment with BRAFi and MEKi because of AEs onset after reaching complete response show that almost half of those patients eventually relapse [36, 37, 46, 47]. Even if most of these patients seem to gain benefit from treatment rechallenge [47, 48], this suggests that therapy continuation should be pursued whenever possible, even in those patients showing complete response to treatment. Notably, there is strong evidence that global health and most functional and symptom domain scores improve significantly in favor of the combination therapy group compared with BRAFi alone [49–51].

Finally, to optimize the efficacy and the different spectrum of toxicity with targeted therapy and immunotherapy, clinical trials are currently underway to elucidate whether sequential and/or interrupting administration of BRAFi and MEKi, also in combination with different treatment approaches (mainly immunotherapy), could optimize disease response and outcomes (see further section).

Perspectives

The combination of BRAFi and MEKi has revolutionized the treatment of patients with metastatic melanoma. However, despite the unquestionable improvement in the response rate and disease control obtained with combined targeted therapies, acquired resistance eventually develops in more than half of patients after approximately 12 months from the beginning of treatment [51]. Significant efforts are ongoing to understand how to obtain the best response by combining BRAFi and MEKi and how to sequence or combine targeted therapy with ICIs. Most importantly, biomarkers and/or clinical features should be identified to select patients with BRAF mutant disease who can benefit more from BRAFi plus MEKi and those who could obtain better disease control with a planned sequence or an upfront combination of ICIs in association with BRAFi and MEKi.

There is plenty of evidence that BRAFi and MEKi have immune-modulatory properties [52]. BRAFi can downregulate immunosuppressive cytokines, decrease the recruitment of regulatory T cells (T regs) and myeloid-derived stem cells (MDSCs), and increase major histocompatibility complex (MHC) class I and antigen expression. Blocking the MAPK pathway in *in vitro* cell lines leads to an increased antigen expression and enhanced reactivity to antigen-specific T lymphocytes [53]. Although in *in vitro* experiments, MEKis may promote a T cell suppressive microenvironment [54, 55], in tumor biopsies from melanoma patients receiving BRAFi and MEKi (either alone or in combination), there is evidence that blocking two steps in the MAPK signaling, the effects are similar on the immunosuppressive microenvironment [55–57].

Despite promising preliminary results, however, most clinical trials investigating the combination of ICIs with targeted therapy failed to demonstrate a signifi-

cant improvement in terms of ORR and survival rate for the triple combination, at the expense of increased toxicity [58, 59]. The only phase-III trial demonstrating a superior PFS for the combination of ICIs and targeted therapy was the IMspire150. In this randomized trial, 514 patients with unresectable stage IIIc-IV, $BRAF^{V600}$ mutation-positive melanoma were randomly assigned 1:1 to atezolizumab, vemurafenib, and cobimetinib or atezolizumab placebo, vemurafenib, and cobimetinib (the control group) [60]. At a median follow-up of 18.9 months, PFS was significantly prolonged with atezolizumab *versus* control (15.1 *vs.* 10.6 months; p = 0.025). The most common treatment-related AEs in the atezolizumab and control groups were increased blood CPK (51.3% *vs.* 44.8%), diarrhoea (42.2% *vs.* 46.6%), rash (40.9%, both groups), arthralgia (39.1% *vs.* 28.1%), pyrexia (38.7% *vs.* 26.0%), increased alanine aminotransferase (33.9% *vs.* 22.8%), and increased lipase (32.2% *vs.* 27.4%). Overall, 13% of patients in the atezolizumab group and 16% in the control group stopped study treatment because of adverse events [60].

In the context of combining targeted therapy with immunotherapies, phase I and I/II studies are investigating the combination of BRAFi + MEKi with new molecules, like heat shock protein 90 inhibitor (Hsp90i) (NCT02721459), colony-stimulating factor 1-receptor inhibitor (CSF-1Ri) (NCT 03101254), and cytokines like IFN and IL-2. Further innovative strategies include the combination of standard therapies (namely BRAFi and chemotherapy) with adoptive cell transfer (ACT) and/or tumor-infiltrating lymphocytes (TIL). Given that such combinations may not be suitable for all patients, in terms of toxicities but also of increased costs, clinical trials are investigating the best sequential regimens of BRAFi + MEKi and ICIs. The rationale behind sequential strategies lies in different kinetics of response between combo-targeted therapy and immunotherapy. Patients with baseline unfavorable prognostic factors (i.e. elevated serum LDH, high tumor burden) are less likely to respond to upfront immunotherapy but could benefit from immunotherapy once LDH levels are normalized and the tumor burden reduced with BRAFi + MEKi-based induction treatment. The SECOMBIT study, a randomized three-arm phase-II study with no formal comparative test (NCT02631447), was started to investigate the best sequential strategy of treatment for patients with BRAF mutant melanoma. In this study, 251 patients were randomized to Arm A (encorafenib plus binimetinib until progressive disease, followed by ipilimumab and nivolumab until progressive disease), or Arm B (ipilimumab and nivolumab until progressive disease, followed by encorafenib plus binimetinib until progressive disease), or Arm C (encorafenib plus binimetinib for 8 weeks, followed by ipilimumab and nivolumab until progressive disease, followed by encorafenib plus binimetinib until progressive disease) [61]. The study primary endpoint of OS was met in each arm; the median OS was not reached in any of the treatment arms. The survival rate at 2 and 3 years was 65% and 54% in arm A, 73% and 62% in arm B, and 69% and 60% in arm C, respectively. Total PFS rate at 2 and 3 years was 46% and 41% in arm A, 65% and 53% in arm B, 57% and 54% in arm C.

Similarly, the DREAMseq study randomized 265 patients with treatment-naïve BRAF V600 positive metastatic melanoma to receive step I treatment with nivolumab plus ipilimumab (arm A) or dabrafenib plus trametinib (arm B). Upon disease progression, patients were enrolled in step II of the trial: patients in arm A switched over to dabrafenib plus trametinib, while patients in arm B switched to nivolumab plus ipilimumab [62]. At a median follow-up of 27.7 months, PFS showed a trend (p = 0.054) favoring patients in arm A. As for OS, a 20% difference in survival was observed (p = 0.0095) at the 2-year time point (72% and 52% for arm A and arm B, respectively) [62]. Even though these preliminary data are interesting, results from these two studies do not consent to do derive significant recommendations to be used in the clinical practice.

Another interesting strategy to synergize the effect of BRAFi +/- MEKi is represented by inhibition of the cyclin-dependent kinase (CDK) 4–6, which is a highly dysregulated pathway in melanoma. Evidence from *in vitro* and *in vivo* studies of the upfront combination of the CDK 4/6 inhibitor palbociclib in combination with BRAFi and/or MEKi seem to evade cell resistance and induce sustained tumor regression [63, 64]. Moreover, co-targeting of MEK and CDK 4/6 seems to have therapeutic effects in a subset of cutaneous melanoma regardless of their mutational status (i.e. NRAS, BRAF mutant, as well as wild-type melanomas) [65]. The use of CDK 4/6 inhibitors in combination with BRAFi and/or MEKi is currently under investigation in ongoing clinical trials [66].

Future clinical trials will include a consistent body of translational research (baseline tissue and plasma samples, with analysis of their dynamic changes during treatment) that will help identify which patients are more likely to gain long-term benefit from sequential or combined targeted and immune therapy.

Conclusions

In the last decade, the medical oncology community has witnessed a dramatic paradigm shift in the treatment of metastatic melanoma. Targeted therapy with BRAFi and MEKi has provided undoubted therapeutic improvement for BRAF mutant disease. However, patients' selection and the onset of acquired resistance during treatment are still problematic. One of the most

fascinating fields of the investigation remains how to integrate immunotherapy with targeted therapies in *BRAF* mutated melanoma patients. There is, indeed, strong evidence now supporting the notion that the therapeutic efficacy of BRAFi and MEKi relies on other factors including the immunomodulation of the microenvironment. Nevertheless, several unanswered questions remain, mostly regarding potential therapeutic combinations and treatment sequencing. Prospective clinical trials are needed to identify the best therapeutic strategy for the treatment of BRAF mutant melanoma and to further improve therapeutic results in this setting.

Conflict of interest

None to declare.

References

1. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009; 27(36): 6199–6206, doi: 10.1200/JCO.2009.23.4799, indexed in Pubmed: 19917835.
2. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363(8): 711–723, doi: 10.1056/NEJMoa1003466, indexed in Pubmed: 20525992.
3. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015; 16(4): 375–384, doi: 10.1016/S1470-2045(15)70076-8, indexed in Pubmed: 25795410.
4. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015; 16(8): 908–918, doi: 10.1016/S1470-2045(15)00083-2, indexed in Pubmed: 26115796.
5. Tsao H, Chin L, Garraway LA, et al. Melanoma: from mutations to medicine. Genes Dev. 2012; 26(11): 1131–1155, doi: 10.1101/gad.191999.112, indexed in Pubmed: 22661227.
6. Rajkumar S, Watson IR. Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. Cell. 2015; 161(7): 1681–1696, doi: 10.1016/j.cell.2015.05.044, indexed in Pubmed: 26091043.
7. Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. Cell. 2012; 150(2): 251–263, doi: 10.1016/j.cell.2012.06.024, indexed in Pubmed: 22817889.
8. Farooq A, Zhou MM. Structure and regulation of MAPK phosphatases. Cell Signal. 2004; 16(7): 769–779, doi: 10.1016/j.cellsig.2003.12.008, indexed in Pubmed: 15115656.
9. Samatar AA, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. Nat Rev Drug Discov. 2014; 13(12): 928–942, doi: 10.1038/nrd4281, indexed in Pubmed: 25435214.
10. Chapman PB, Hauschild A, Robert C, et al. BRIM-3 Study Group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011; 364(26): 2507–2516, doi: 10.1056/NEJMoa1103782, indexed in Pubmed: 21639808.
11. McArthur GA, Chapman PB, Robert C, et al. Safety and efficacy of vemurafenib in BRAF(V600E) and BRAF(V600K) mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. Lancet Oncol. 2014; 15(3): 323–332, doi: 10.1016/S1470-2045(14)70012-9, indexed in Pubmed: 24508103.
12. Chapman PB, Robert C, Larkin J, et al. Vemurafenib in patients with BRAFV600 mutation-positive metastatic melanoma: final overall survival results of the randomized BRIM-3 study. Ann Oncol. 2017; 28(10): 2581–2587, doi: 10.1093/annonc/mdx339, indexed in Pubmed: 28961848.
13. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012; 380(9839): 358–365, doi: 10.1016/S0140-6736(12)60868-X, indexed in Pubmed: 22735384.
14. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. Nat Rev Clin Oncol. 2014; 11(7): 385–400, doi: 10.1038/nrclinonc.2014.83, indexed in Pubmed: 24840079.
15. Lito P, Rosen N, Solit D. Tumor adaptation and resistance to RAF inhibitors. Nat Med. 2013; 19(11): 1401–1409, doi: 10.1038/nm.3392.
16. Trunzer K, Pavlick AC, Schuchter L, et al. Pharmacodynamic effects and mechanisms of resistance to vemurafenib in patients with metastatic melanoma. J Clin Oncol. 2013; 31(14): 1767–1774, doi: 10.1200/JCO.2012.44.7888, indexed in Pubmed: 23569304.
17. Villanueva J, Vultur A, Lee JT, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell. 2010; 18(6): 683–695, doi: 10.1016/j.ccr.2010.11.023, indexed in Pubmed: 21156289.
18. Wongchenko MJ, McArthur GA, Dréno B, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med. 2014; 371(20): 1867–1876, doi: 10.1056/NEJMoa1408868, indexed in Pubmed: 25265494.
19. Long GV, Stroyakovskiy D, Gogas H, et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. Lancet. 2015; 386(9992): 444–451, doi: 10.1016/S0140-6736(15)60898-4, indexed in Pubmed: 26037941.
20. Dummer R, Ascierto PA, Gogas HJ, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2018; 19(5): 603–615, doi: 10.1016/S1470-2045(18)30142-6, indexed in Pubmed: 29573941.
21. Ribas A, Gonzalez R, Pavlick A, et al. Combination of vemurafenib and cobimetinib in patients with advanced BRAFV600-mutated melanoma: a phase 1b study. Lancet Oncol. 2014; 15(9): 954–965, doi: 10.1016/s1470-2045(14)70301-8.
22. Ascierto PA, McArthur GA, Dréno B, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol. 2016; 17(9): 1248–1260, doi: 10.1016/S1470-2045(16)30122-X, indexed in Pubmed: 27480103.
23. Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med. 2012; 367(18): 1694–1703, doi: 10.1056/NEJMoa1210093, indexed in Pubmed: 23020132.
24. Long GV, Stroyakovskiy D, Gogas H, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med. 2014; 371(20): 1877–1888, doi: 10.1056/NEJMoa1406037, indexed in Pubmed: 25265492.
25. Robert C, Grob JJ, Stroyakovskiy D, et al. Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma. N Engl J Med. 2019; 381(7): 626–636, doi: 10.1056/NEJMoa1904059, indexed in Pubmed: 31166680.
26. Long GV, Flaherty KT, Stroyakovskiy D, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. Ann Oncol. 2017; 28(7): 1631–1639, doi: 10.1093/annonc/mdx176, indexed in Pubmed: 28475671.
27. Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med. 2015; 372(1): 30–39, doi: 10.1056/NEJMoa1412690, indexed in Pubmed: 25399551.
28. Robert C, Karaszewska B, Schachter J, et al. Three-year estimate of overall survival in COMBI-v, a randomized phase 3 study evaluating first-line dabrafenib (D) + trametinib (T) in patients (pts) with unresectable or metastatic BRAF V600E/K–mutant cutaneous melanoma. Ann Oncol. 2016; 27: vi575, doi: 10.1093/annonc/mdw435.37.
29. Kim KB, Kefford R, Pavlick AC, et al. Phase II study of the MEK1/MEK2 inhibitor Trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. J Clin Oncol. 2013; 31(4): 482–489, doi: 10.1200/JCO.2012.43.5966, indexed in Pubmed: 23248257.
30. Flaherty KT, Robert C, Hersey P, et al. METRIC Study Group. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med. 2012; 367(2): 107–114, doi: 10.1056/NEJMoa1203421, indexed in Pubmed: 22663011.
31. Dummer R, Ascierto PA, Gogas HJ, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2018; 19(5): 603–615, doi: 10.1016/S1470-2045(18)30142-6, indexed in Pubmed: 29573941.
32. Dummer R, Ascierto PA, Gogas H, et al. Results of COLUMBUS Part 2: A phase 3 trial of encorafenib (ENCO) plus binimetinib (BINI) versus ENCO in BRAF-mutant melanoma. Ann Oncol. 2017; 28: v429–v430, doi: 10.1093/annonc/mdx377.002.

33. Dummer R, Flaherty K, Robert C, et al. Five-year overall survival (OS) in COLUMBUS: A randomized phase 3 trial of encorafenib plus binimetinib versus vemurafenib or encorafenib in patients (pts) with BRAF V600--mutant melanoma. J Clin Oncol. 2021; 39(15_suppl): 9507–9507, doi: 10.1200/jco.2021.39.15_suppl.9507.
34. Long GV, Grob JJ, Nathan P, et al. Factors predictive of response, disease progression, and overall survival after dabrafenib and trametinib combination treatment: a pooled analysis of individual patient data from randomised trials. Lancet Oncol. 2016; 17(12): 1743–1754, doi: 10.1016/S1470-2045(16)30578-2, indexed in Pubmed: 27864013.
35. Schadendorf D, Long GV, Stroiakovski D, et al. Three-year pooled analysis of factors associated with clinical outcomes across dabrafenib and trametinib combination therapy phase 3 randomised trials. Eur J Cancer. 2017; 82: 45–55, doi: 10.1016/j.ejca.2017.05.033, indexed in Pubmed: 28648698.
36. Carlino MS, Vanella V, Girgis C, et al. Cessation of targeted therapy after a complete response in BRAF-mutant advanced melanoma: a case series. Br J Cancer. 2016; 115(11): 1280–1284, doi: 10.1038/bjc.2016.321, indexed in Pubmed: 27711085.
37. Desvignes C, Abi Rached H, Templier C, et al. BRAF inhibitor discontinuation and rechallenge in advanced melanoma patients with a complete initial treatment response. Melanoma Res. 2017; 27(3): 281–287, doi: 10.1097/CMR.0000000000000350, indexed in Pubmed: 28240681.
38. Chan MMK, Haydu LE, Menzies AM, et al. The nature and management of metastatic melanoma after progression on BRAF inhibitors: effects of extended BRAF inhibition. Cancer. 2014; 120(20): 3142–3153, doi: 10.1002/cncr.28851, indexed in Pubmed: 24985732.
39. Puzanov I, Amaravadi RK, McArthur GA, et al. Long-term outcome in BRAF(V600E) melanoma patients treated with vemurafenib: Patterns of disease progression and clinical management of limited progression. Eur J Cancer. 2015; 51(11): 1435–1443, doi: 10.1016/j.ejca.2015.04.010, indexed in Pubmed: 25980594.
40. Long GV, Trefzer U, Davies MA, et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK--MB): a multicentre, open-label, phase 2 trial. Lancet Oncol. 2012; 13(11): 1087–1095, doi: 10.1016/S1470-2045(12)70431-X, indexed in Pubmed: 23051966.
41. Davies MA, Saiag P, Robert C, et al. Dabrafenib plus trametinib in patients with BRAF-mutant melanoma brain metastases (COMBI-MB): a multicentre, multicohort, open-label, phase 2 trial. Lancet Oncol. 2017; 18(7): 863–873, doi: 10.1016/S1470-2045(17)30429-1, indexed in Pubmed: 28592387.
42. Marquez-Rodas I, Arance A, Guerrero MAB, et al. 1038MO Intracranial activity of encorafenib and binimetinib followed by radiotherapy in patients with BRAF mutated melanoma and brain metastasis: Preliminary results of the GEM1802/EBRAIN-MEL phase II clinical trial. Ann Oncol. 2021; 32: S870, doi: 10.1016/j.annonc.2021.08.1423.
43. Dréno B, Ribas A, Larkin J, et al. Incidence, course, and management of toxicities associated with cobimetinib in combination with vemurafenib in the coBRIM study. Ann Oncol. 2017; 28(5): 1137–1144, doi: 10.1093/annonc/mdx040, indexed in Pubmed: 28444112.
44. Urner-Bloch U, Urner M, Jaberg-Bentele N, et al. Transient MEK inhibitor-associated retinopathy in metastatic melanoma. Ann Oncol. 2014; 25(7): 1437–1441, doi: 10.1093/annonc/mdu169, indexed in Pubmed: 24864047.
45. Carlos G, Anforth R, Clements A, et al. Cutaneous Toxic Effects of BRAF Inhibitors Alone and in Combination With MEK Inhibitors for Metastatic Melanoma. JAMA Dermatol. 2015; 151(10): 1103–1109, doi: 10.1001/jamadermatol.2015.1745, indexed in Pubmed: 26200476.
46. Tolk H, Satzger I, Mohr P, et al. Complete remission of metastatic melanoma upon BRAF inhibitor treatment - what happens after discontinuation? Melanoma Res. 2015; 25(4): 362–366, doi: 10.1097/CMR.0000000000000169, indexed in Pubmed: 26061438.
47. Schreuer M, Jansen Y, Planken S, et al. Combination of dabrafenib plus trametinib for BRAF and MEK inhibitor pretreated patients with advanced BRAF-mutant melanoma: an open-label, single arm, dual-centre, phase 2 clinical trial. Lancet Oncol. 2017; 18(4): 464–472, doi: 10.1016/S1470-2045(17)30171-7, indexed in Pubmed: 28268064.
48. Valpione S, Carlino MS, Mangana J, et al. Rechallenge with BRAF-directed treatment in metastatic melanoma: A multi-institutional retrospective study. Eur J Cancer. 2018; 91: 116–124, doi: 10.1016/j.ejca.2017.12.007, indexed in Pubmed: 29360604.
49. Grob JJ, Amonkar MM, Karaszewska B, et al. Comparison of dabrafenib and trametinib combination therapy with vemurafenib monotherapy on health-related quality of life in patients with unresectable or metastatic cutaneous BRAF Val600-mutation-positive melanoma (COMBI-v): results of a phase 3, open-label, randomised trial. Lancet Oncol. 2015; 16(13): 1389–1398, doi: 10.1016/S1470-2045(15)00087-X, indexed in Pubmed: 26433819.
50. Schadendorf D, Amonkar MM, Stroyakovskiy D, et al. Health-related quality of life impact in a randomised phase III study of the combination of dabrafenib and trametinib versus dabrafenib monotherapy in patients with BRAF V600 metastatic melanoma. Eur J Cancer. 2015; 51(7): 833–840, doi: 10.1016/j.ejca.2015.03.004, indexed in Pubmed: 25794603.
51. Rizos H, Menzies AM, Pupo GM, et al. BRAF inhibitor resistance mechanisms in metastatic melanoma: spectrum and clinical impact. Clin Cancer Res. 2014; 20(7): 1965–1977, doi: 10.1158/1078-0432.CCR-13-3122, indexed in Pubmed: 24463458.
52. Mandalà M, De Logu F, Merelli B, et al. Immunomodulating property of MAPK inhibitors: from translational knowledge to clinical implementation. Lab Invest. 2017; 97(2): 166–175, doi: 10.1038/labinvest.2016.132, indexed in Pubmed: 27991907.
53. Boni A, Cogdill AP, Dang P, et al. Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. Cancer Res. 2010; 70(13): 5213–5219, doi: 10.1158/0008-5472.CAN-10-0118, indexed in Pubmed: 20551059.
54. Ott PA, Henry T, Baranda SJ, et al. Inhibition of both BRAF and MEK in BRAF(V600E) mutant melanoma restores compromised dendritic cell (DC) function while having differential direct effects on DC properties. Cancer Immunol Immunother. 2013; 62(4): 811–822, doi: 10.1007/s00262-012-1389-z, indexed in Pubmed: 23306863.
55. Wilmott JS, Long GV, Howle JR, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res. 2012; 18(5): 1386–1394, doi: 10.1158/1078-0432.CCR-11-2479, indexed in Pubmed: 22156613.
56. Frederick DT, Piris A, Cogdill AP, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res. 2013; 19(5): 1225–1231, doi: 10.1158/1078-0432.CCR-12-1630, indexed in Pubmed: 23307859.
57. Kakavand H, Wilmott JS, Menzies AM, et al. PD-L1 Expression and Tumor-Infiltrating Lymphocytes Define Different Subsets of MAPK Inhibitor-Treated Melanoma Patients. Clin Cancer Res. 2015; 21(14): 3140–3148, doi: 10.1158/1078-0432.CCR-14-2023, indexed in Pubmed: 25609064.
58. Ferrucci PF, Di Giacomo AM, Del Vecchio M, et al. KEYNOTE-022 international team. KEYNOTE-022 part 3: a randomized, double-blind, phase 2 study of pembrolizumab, dabrafenib, and trametinib in -mutant melanoma. J Immunother Cancer. 2020; 8(2), doi: 10.1136/jitc-2020-001806, indexed in Pubmed: 33361337.
59. Dummer R, Long GV, Robert C, et al. Randomized Phase III Trial Evaluating Spartalizumab Plus Dabrafenib and Trametinib for V600--Mutant Unresectable or Metastatic Melanoma. J Clin Oncol. 2022 [Epub ahead of print]: JCO2101601, doi: 10.1200/JCO.21.01601, indexed in Pubmed: 35030011.
60. Gutzmer R, Stroyakovskiy D, Gogas H, et al. Atezolizumab, vemurafenib, and cobimetinib as first-line treatment for unresectable advanced BRAF mutation-positive melanoma (IMspire150): primary analysis of the randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2020; 395(10240): 1835–1844, doi: 10.1016/S0140--6736(20)30934-X, indexed in Pubmed: 32534646.
61. Ascierto PA, Mandala M, Ferrucci PF, et al. LBA40 SECOMBIT: The best sequential approach with combo immunotherapy [ipilimumab (I) /nivolumab (N)] and combo target therapy [encorafenib (E)/binimetinib (B)] in patients with BRAF mutated metastatic melanoma: A phase II randomized study. Ann Oncol. 2021; 32: S1316–S1317, doi: 10.1016/j.annonc.2021.08.2118.
62. Atkins MB, Lee SJ, Chmielowski B, et al. DREAMseq: A phase III trial – ECOG Acrin ECOG-ACRIN EA6134. ASCO Plenary Series. Abstract 356154. Presented November 16, 2021.
63. Martin CA, Cullinane C, Kirby L, et al. Palbociclib synergizes with BRAF and MEK inhibitors in treatment naïve melanoma but not after the development of BRAF inhibitor resistance. Int J Cancer. 2018; 142(10): 2139–2152, doi: 10.1002/ijc.31220, indexed in Pubmed: 29243224.
64. Lee B, McArthur GA. CDK4 inhibitors an emerging strategy for the treatment of melanoma. Melanoma Manag. 2015; 2(3): 255–266, doi: 10.2217/mmt.15.14, indexed in Pubmed: 30190853.
65. Posch C, Sanlorenzo M, Ma J, et al. MEK/CDK4,6 co-targeting is effective in a subset of NRAS, BRAF and 'wild type' melanomas. Oncotarget. 2018; 9(79): 34990–34995, doi: 10.18632/oncotarget.26204, indexed in Pubmed: 30405888.
66. Taylor M, Sosman J, Gonzalez R, et al. Phase Ib/Ii Study of Lee011 (Cdk4/6 Inhibitor) and Lgx818 (Braf Inhibitor) in Braf-Mutant Melanoma. Ann Oncol. 2014; 25: iv374, doi: 10.1093/annonc/mdu344.2.